# SYNOPSIS

## PROTECTIVE EFFICACY OF OMEGA-3 FATTY ACID AGAINST ARSENIC TRIOXIDE (AN ANTI-LEUKEMIC AGENT) INDUCED TOXICITY

Modern chemotherapeutic drugs have improved the efficiency of cancer therapy, but their use is hindered by toxicity to healthy tissue. Prakash et al (2010) has reported that, more than three fourths of deaths were related to chemotherapeutic side effects in cancer patients. Chemotherapeutic agents comprise of anthracyclines, antimetabolites, metalloids, alkylating agents, and antimicrotubule substances. They have showed severe side effects, not only during treatment but also in the long run (Albini et al, 2010). Production of hydroxyl free radicals is associated with anti-tumor effects and toxicity to healthy tissues. The reported serious side effects are myocardial, hepatic and renal complications, which can span from subclinical abnormalities to serious life-threatening events (Fong and Bentrem, 2006; Sahni et al, 2009; Yeh and Bickford, 2009).

Arsenic is a metalloid which has gained worldwide attention because of its substantial anticancer activity (Sekhon, 2013). The use of arsenic trioxide ($As_2O_3$) to treat leukaemia began at the Harbin Medical University in the 1970s. In September 2000, the *Trisenox* brand of $As_2O_3$ for the treatment of relapsed and refractory acute promyelocytic leukemia (APL) was approved by the Food and Drug Administration (FDA), USA (Cohen et al, 2001). Arsenic-mediated cell death was not restricted to APL but holds to other malignant cells, including non-APL acute myeloid leukemia cells, myeloma cells, chronic myeloid leukemia cells and solid-tumor cells of esophageal, prostate, ovarian and neuroblastoma origin (Cheng et al, 2010). $As_2O_3$ induces a high remission rate in patients with both primary and relapsed APL (around 85% to 90%) (Chen et al, 2001). Myocardial tissue is particularly susceptible to free radical damage by $As_2O_3$ exposure. Electrocardiography in patients receiving $As_2O_3$ has shown significant QT interval prolongation in 35% of cases, with symptomatic torsades de pointes in 1–3% of cases (Barbey et al, 2003). The $As_2O_3$ treatment in cancer patients showed hepatotoxic side effects and renal failure (Majumdar et al, 2012; Yamamoto and Goodman, 2009). Sudden death was also reported among APL patients undergoing $As_2O_3$ treatment (Westervelt et al, 2001). So there is a need for the identification of an effective compound, which reduces the toxic effects of $As_2O_3$.

Many epidemiological studies and clinical trials have shown a beneficial relationship of omega-3 fatty acid with cardiovascular health, hepatic functions, anticancer properties and inflammatory diseases (Simopoulos, 2003; Gómez-Candela et al, 2011). Fish oil mainly contains omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which beneficially reduced arrhythmic risks and protected from sudden cardiac death (Kris-Etherton et al, 2003). Omega-3 fatty acid supplementation prevents alterations in hepatic steatosis, hepatic pathology and modified liver functions (Alwayn et al, 2005; Davidson, 2006). DHA and EPA reduce inflammation in renal tissues and maintain renal functions (Katakura et al, 2014). Perceiving the above health benefits, omega-3 fatty acid was selected for studying its effectiveness against $As_2O_3$ induced cardiac, hepatic and renal toxicity. The main objective of the current investigation was to evaluate the efficacy of omega-3 fatty acid against the chemotherapeutic agent $As_2O_3$ induced toxicity.

The extent of toxic potential of $As_2O_3$ was assessed with experimental model - male albino Wistar rats by supplementing $As_2O_3$ (2, 4 & 8 mg/kg body weight), orally for 45 days. The inductively coupled plasma optical emission spectroscopic analysis revealed arsenic deposition in heart, liver and kidney. Increased release of cardiac, hepatic and renal marker enzymes were identified in systemic circulation. Blood glucose, serum potassium and calcium concentrations were altered with $As_2O_3$ treatment. $As_2O_3$ treatment resulted in significant depletion in the antioxidant defence mechanism. The lipid peroxidation in blood and tissues were significantly increased. Histopathological examination revealed structural abnormalities in cardiac, hepatic and renal tissues subjected to $As_2O_3$ treatment.

The protective effect of omega-3 fatty acid was assessed by combination treatment with $As_2O_3$. Two concentrations of omega-3 fatty acid (25mg and 50mg/kg body weight) were used for identifying the effective concentration against $As_2O_3$ (4mg/kg body weight) induced toxic effects. Omega-3 fatty acid co-treatment effectively reduced the arsenic deposition in heart, liver and kidney. A reduction in the cardiac, hepatic and renal marker enzymes was also noticed. Omega-3 fatty acid co-treatment significantly controlled the blood glucose, serum potassium and calcium concentrations. Antioxidant enzymes were significantly increased and the lipid peroxidation was found reduced. The structural aberrations of cardiac, hepatic and renal tissues were significantly decreased with omega-3 fatty acid co-treatment. From all the observations,

omega-3 fatty acid concentration 50mg/kg body weight was found to be more effective than the concentration of 25mg/kg body weight against $As_2O_3$ toxicity.

Cardiac conducting mechanism was assessed by electrocardiographic (ECG) analysis. Omega-3 fatty acid co-treatment significantly reduced the $As_2O_3$ induced ECG alterations (QT and QTc intervals) by its anti-arrhythmic action. Cytokinesis block micronuclei assay revealed that the co-treatment with omega-3 fatty acid reduced the arsenic induced micronuclei frequency, reflecting its protection against the $As_2O_3$ induced genotoxicity. Transmission electron microscopic analysis shows that omega-3 fatty acid improved the $As_2O_3$ induced cardiac ultrastructural changes-maintained cell membrane integrity, reduced interstitial edema, and the structural aberrations of mitochondrial and myofilaments. The findings of the current investigation suggest that the omega-3 fatty acid is an effective cardio protective agent against $As_2O_3$ toxicity.

*In vitro* experiments were performed in H9c2 cardiomyocytes to identify the cellular mechanism of cardio protection. The effective concentration of docosahexaenoic acid (DHA) (100µM) was identified by combination of DHA (50,100 & 150µM) with $As_2O_3$ (10µM) by cell viability assay. $As_2O_3$-treatement increased lactate dehydrogenase (LDH) release, lipid peroxidation, cellular calcium ($Ca^{2+}$) levels and decreased mitochondrial membrane potential ($\Delta\Psi m$). Cardioprotective efficacy of omega-3 fatty acid was assessed by the combination treatment with $As_2O_3$. Omega-3 fatty acid co-treatment significantly reduced the LDH release, lipid peroxidation, intracellular $Ca^{2+}$ concentration and improved the $\Delta\Psi m$. These *in vitro* findings suggested that the omega-3 fatty acids can reduce the $As_2O_3$ induced toxic cellular changes in cardiomyocytes.

The effect of omega-3 fatty acid co-treatment on cancer cells was assessed with human acute promyelocytic leukemia cell line HL-60. Omega-3 fatty acid co-treatment increased the $As_2O_3$ induced cytotoxicity by increasing the LDH release, lipid peroxidation, intracellular $Ca^{2+}$ level and reduced $\Delta\Psi m$. The reduced $\Delta\Psi m$ may result in the activation of mitochondrial permeability transition pore opening and there by the release of apoptotic factors in to the cytoplasm, leading to cell death. The results of the current *in vitro* investigation suggest that omega-3 fatty acid treatment during $As_2O_3$ therapy can increase the effectiveness of chemotherapy.

The findings of the current *in vivo* and *in vitro* research advocate that, the supplementation of omega-3 fatty acid during chemotherapy could be an effectual option for reducing the toxic effects persuaded by $As_2O_3$. The outcome of this study can be used to develop a combination treatment strategy or a nutritional supplementation plan to patients undergoing APL treatment.

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